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Phytochemical composition and anti-nutritional factors of *Senna occidentalis* dry seeds

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Abstract
False kinkeliba (*Senna occidentalis*) is a species found throughout Africa and on other continents. Previous studies on this plant have shown its great potential in various fields. The aim of this study was to evaluate phytochemical content and antinutritional factors of this plant's seeds. Seeds were removed from dry pods and ground into powder. Conventional biochemical analysis methods were used to evaluate antioxidant capacity, phenolic compound content, anti-nutrient content and heavy metal composition of dry seeds powder. Results indicate that sample is rich in bioactive compounds, with total polyphenol content (233.33 ± 0.02 mg EAG. g^{-1}), flavonoids (360 ± 0.01 mg EQ. g^{-1}) and total tannins (586.70 ± 14.91 mg EAT. g^{-1}). Phytate and oxalate contents are 71.61 ± 1.84 mg. 100 g^{-1} and 73.33 ± 0.03 mg. 100 g^{-1}, respectively. All heavy metals detected, such as lead (0.026 mg. kg^{-1}), mercury (0.009 mg. kg^{-1}) and cadmium (0.010 mg. kg^{-1}), have concentrations well below the tolerable threshold for human consumption.

Keywords: *Senna occidentalis*, Seeds, antioxidants, anti-nutrients, Côte d'Ivoire

Introduction

In Africa, plant diversity is of paramount importance to the population [1, 2]. In most cases, plants are used to meet many daily needs, including nutritional, medicinal and cosmetic requirements [3, 4, 5]. Studies conducted on different parts of these species show the high potential of natural substances they contain [6, 7, 8]. With regard to *Senna occidentalis* (commonly known as false kinkeliba or dartrier), several studies conducted around the world have focused on aerial and root parts [9, 10]. Toxicological research has shown that its seeds contain a substance that may be responsible for mitochondrial myopathy [11, 12]. In Côte d'Ivoire, seeds of this plant are traditionally used in several regions to produce a beverage that is a perfect substitute for coffee [13]. However, there is virtually no literature highlighting the impact of this consumption on health safety of those who use it. Thus, this work, which forms part of efforts to promote local food species, aims to lay the scientific foundations for assessing secondary metabolite and anti-nutrient content of *Senna occidentalis* seeds from Côte d'Ivoire.

Materials and Methods

Plant material

Plant material used for this study consists mainly of dried *Senna occidentalis* seeds that have been ground into powder (Figure 1). Seeds were extracted from pods that had dried on plants. Pods were harvested during March, April and May 2025 in Korhogo (a town in northern Côte d'Ivoire). This town is geographically located at coordinates 9° 27′ 41″ north and 5° 38′ 19″ west. It is characterised by high interannual variability in rainfall, with averages of around 1343 mm per year. Its climate is Sudano-Guinean, with a long dry season and a long rainy season, in a wooded savannah environment.

Fig 1: A- dry seeds and B- powder from dry seeds of *Senna occidentalis*

Methods

Determination of total antioxidant activity

The ability to scavenge DPPH (2,2-diphenyl-1-picrylhydrazyl) radical was assessed using method described in [14]. One (1) g of sample was homogenised in 10 ml of methanol (70 %), then centrifuged at 1000 rpm for 10 min. In a test tube containing 2.5 ml of supernatant, 1 ml of DPPH solution (3 mM in methanol) was added. The mixture was left to stand for 30 min in dark and absorbance was read at 415 nm using a spectrophotometer. Antioxidant activity value (AA) expressed as % relative inhibition was estimated using following calculation (1):

$$AA\ (\%) = \frac{[\text{Abs control} - \text{Abs sample}]}{\text{Abs control}} \times 100 \qquad (1)$$

With Abs control: absorbance of control tube
Abs sample: absorbance of test tube

Determination of total polyphenols

Total polyphenols were measured using colorimetric method described in [15]. In a test tube, 2.5 ml of Folin-Ciocalteu reagent diluted to 1/10th was added to 30 µl of extract. This mixture was left in dark for 2 minutes at room temperature (30 ± 2 °C). Next, 2 ml of 7.5 % sodium carbonate solution was added. This mixture was placed in a water bath, maintained at 50 °C for 15 min, then cooled rapidly. Absorbance was measured using a UV/visible spectrophotometer at a wavelength of 760 nm with distilled water as blank. Gallic acid was used as standard. Total polyphenol content of extract was determined using calibration curve of gallic acid at different concentrations (0 to 1.4 mg. ml^{-1}). Its value (Qpolyph) expressed in milligrams of gallic acid equivalent per gram of dry matter (mg EAG. g^{-1} DM) was calculated according to equation (2) below:

$$Q_{polyph}(mg\ EAG.g^{-1}) = \frac{Conc_{spect} \times DF}{Conc_{ext}} \qquad (2)$$

With Q_{polyph}: total polyphenol content,
$Conc_{spect}$: concentration read by spectrophotometer,
$Conc_{ext}$: concentration of the prepared extract (5 mg. ml^{-1})
DF: dilution factor

Determination of total flavonoids

Total flavonoid content was determined using aluminium chloride colorimetric method as described by [16]. In a 25 ml flask, 2.5 ml of extract solution prepared at 5 mg. ml^{-1} was added to 0.75 ml of sodium nitrite ($NaNO_2$ at 5 % (m.v-1)). To this mixture, 0.75 ml of aluminium chloride (10 % $AlCl_3$ (m. v-1)) was added, and the mixture was then placed in the dark for incubation for 6 min. After incubation, 5 ml of sodium hydroxide (1 N NaOH) was added and volume was made up to 25 ml. After vigorous stirring of mixture, absorbance was measured using an UV/visible spectrophotometer at a wavelength of λ = 510 nm against a blank consisting of distilled water. Quercetin was used as standard. Total flavonoid content of extract was obtained using quercetin calibration curve established from different concentrations (0 to 0.9 g. l^{-1}). Total flavonoid content (Q_{flavo}) expressed in milligrams of quercetin equivalent per gram of dry matter (mg EQ. g^{-1} DM) was obtained using the following equation (3):

$$Q_{flavo}(mg\ EQ.g^{-1}) = \frac{Conc_{spect} \times DF}{Conc_{ext}} \qquad (3)$$

With Q_{flavo}: total flavonoid content,
$Conc_{spect}$: concentration read by spectrophotometer,
Concext: concentration of the prepared extract (5 mg. ml^{-1})
DF: dilution factor

Determination of total tannins

Total tannins were measured using method described in [17]. 100 µl of extract solution (5 mg. ml^{-1}) was added to a test tube containing 7.5 ml of distilled water and 0.5 ml of Folin-Ciocalteu reagent; followed by 1 ml of 35 % Na_2CO_3. Volume was made up to 10 ml by adding 900 µl of distilled water. Reaction mixture was stirred and then left to incubate for 30 min at laboratory temperature (25 to 30 °C). Absorbances were read at 700 nm using a UV/visible spectrophotometer against distilled water used as a blank. A calibration curve was plotted from a range of tannic acid concentrations (0; 1.95; 3.9; 7.81; 15.62; 31.25; 62.5; 125; 250; 500 and 1000 µg. ml^{-1}) used as a standard. Total tannin content (Q_{tan}) expressed in micrograms of tannic acid equivalent per gram of dry matter (mg EAT. g^{-1} DM) was calculated from the relationship (4) below:

$$Q_{tan}(mg\ EAT.g^{-1}) = \frac{Conc_{spect} * DF}{Conc_{ext}} \qquad (4)$$

With Qt_{an}: total tannin content,
$Conc_{spect}$: concentration read by spectrophotometer,
$Conc_{ext}$: concentration of the prepared extract (5 mg. ml^{-1})
DF: dilution factor

Determination of condensed tannins

Condensed tannins content was assessed using method described in [18]. In a test tube containing 50 µl of extract solution (5 mg. ml-1), 3 ml of a 4% vanillin methanic acid solution was added, followed by 1.5 ml of 37 % concentrated hydrochloric acid (HCl). Mixture was incubated in dark for 15 minutes at laboratory temperature (25 to 30 °C). Absorbance was then read at 500 nm using a UV/visible spectrophotometer against a blank consisting of MeOH. Catechin was used as standard. Condensed tannins content of extracts was determined according to catechin calibration curve established from different concentrations (0; 1.95; 3.91; 7.81; 15.62; 31.25; 62.5; 125; 250; 500 and 1000 µg. ml^{-1}). Result (Q_{tanC}) expressed in milligrams of catechin equivalent per gram of dry matter (mg EC. g^{-1} DM) was calculated using the following equation (5):

$$Q_{tanC}(mg\ EC.g^{-1}) = \frac{Conc_{spect} \times DF}{Conc_{ext}} \qquad (5)$$

With QtanC: condensed tannin content,

$Conc_{spect}$: concentration read by spectrophotometer,
$Conc_{ext}$: concentration of the prepared extract (5 mg. ml^{-1})
DF: dilution factor

Determination of phytates

Method used to determine phytates content of seeds was that described in [19]. It consisted of homogenising 0.5 g of sample in 10 ml of HCl (0.65 N) with stirring for 12 hours at room temperature. The mixture was then centrifuged at 3000 rpm for 40 min. A volume of 0.5 ml of supernatant was taken and added to 3 ml of Wade's reagent. Mixture was left to stand for 15 min and concentration was read on a spectrophotometer at 490 nm against the blank. A calibration range was performed with sodium phytates at 10 mg/ml and 50 mg. ml^{-1}. Phytates concentration ($Conc_{phyt}$) expressed in milligrams per 100 grams of dry matter (mg. 100 g^{-1} DM) was calculated using the following formula (6):

$$Conc_{phyt} = \frac{Q \times DF}{Pe} \times 100 \quad (6)$$

Where Q: quantity of phytates in each tube (mg)
DF: dilution factor
Pe: test sample

Determination of oxalates

Method used to quantify oxalates was that described by [20]. It consisted of homogenising 1 g of sample in 60 ml of sulphuric acid (3 N) under magnetic stirring for 1 hour. Mixture was filtered through Whatman filter paper. Then, 25 ml of the filtrate was titrated hot with a 0.05 N potassium permanganate ($KMnO_4$) solution until a persistent pink colour appeared. Oxalates content (Q_{oxa}) expressed in milligrams per 100 grams of dry matter (mg. 100 g^{-1} DM) was determined according to formula (7) below:

$$Q_{oxa} = \frac{2{,}2 \times V_{eq}}{P_e} \times 100 \quad (7)$$

Where Veq: volume (ml) of $KMnO_4$ added at equivalence
P_e: test sample.

Determination of heavy metals

These minerals were determined using inductively coupled plasma emission spectrometry (ICP OES, Agilent 5800) according to the method described in [21]. A mass of 0.3 g of dry sample a was calcined at 600 °C for 5 hours in an oven until a white ash was obtained. After cooling, 5 ml of nitric acid (1 N) was added and then evaporated to dryness on a sand bath (or hot plate). Five millilitres of hydrochloric acid (1 N) were added to the residue and the mixture was placed back in the oven at 400 °C for 30 minutes. When calcined residue was removed from the oven, 10 ml of 0.1 N hydrochloric acid were added to crucible to recover the product. Mixture obtained was poured directly into a 50 ml volumetric flask. Flask was filled to mark. After decanting, supernatant was filtered using 0.45 µ Wattman paper. The elements contained in solution were then measured by ICP OES. To avoid interference from Ca and K elements, lanthanum chloride (5 ml of lanthanum) was added.

Results and discussion

Phenolic compounds and total antioxidant activity of *Senna occidentalis* dry seeds: Dietary phytochemicals are a diverse group of compounds that are biologically and physiologically active and have positive health effects [22]. Results obtained from our work have shown that *Senna occidentalis* dry seeds powder occupies a prominent place in diet due to its richness in bioactive elements. Figure 2 highlights values obtained after quantitative determination of total polyphenols, flavonoids, total tannins, condensed tannins and total antioxidant activity.

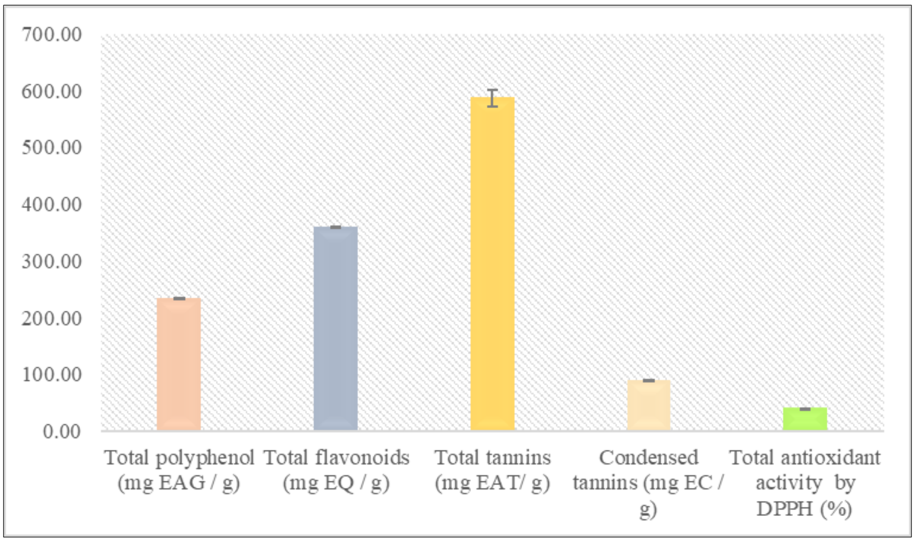

Fig 2: Phenolic compound content and total antioxidant activity of *Senna occidentalis* dry seeds

We noted total polyphenol content of 233.33 ± 0.02 mg EAG. g^{-1}. This amount is significantly higher than that found in defatted lentil seeds (*Lens culinaris*), common beans (*Vicia faba*) and 23 edible legumes from China [23, 24, 25].

For total flavonoids, concentration was 360 ± 0.01 mg EQ. g^{-1}. This content is higher than that of quinoa seeds (*Chenopodium quinoa* Willd) and black cowpea (*Vigna unguiculata*) [26, 27].

For total tannins, value of 586.70 ± 14.91 mg EAT. g^{-1} was recorded. This content is higher than that of sesame seed flour [28]. This makes these seeds beneficial for cardiovascular health and for fighting inflammation.

As for condensed tannins, content obtained was 89.50 ± 0.41 mg EC. g^{-1} of seeds powder. This proportion is higher

than content recorded for two (2) varieties of millet (*Pennisetum glaucum*) studied by [22] and for peanut seeds (*Arachis hypogaea* L.) according to the work of [29].
Total antioxidant activity recorded a value of 39.52 ± 0.02 %, which is higher than that of quinoa (*Chenopodium quinoa* Willd) and roughly equal to that of jujube pulp (*Ziziphus spina-christi* Willd) [26, 30].
According to [31, 32], phytochemical compounds concentration varies from one plant to another and may be strongly influenced by genotype, soil, and environmental conditions.

Phytates and oxalates content of *Senna occidentalis* dry seeds

Based on their metal chelating and enzyme inhibiting properties, certain compounds such as phytates and oxalates are considered antinutritional factors [33]. Phytates and oxalates contents of *Senna occidentalis* dry seeds powder obtained after various analyses are shown in Figure 3.

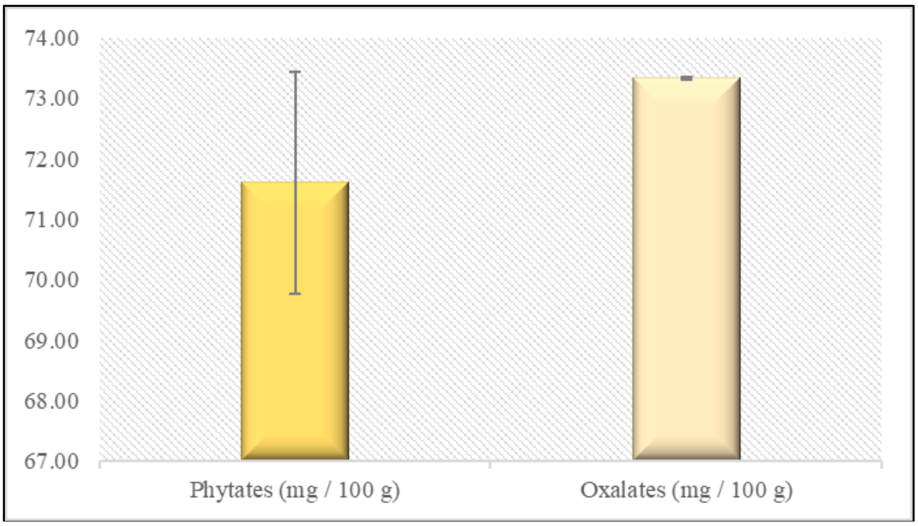

Fig 3: Phytate and oxalate content *Senna occidentalis* dry seeds

For 100 g of sample, phytates's proportion was 71.61 ± 1.84 mg. This amount is very low compared to that of elephant grass (*Eleusine coracana* L.), common bean (*Vicia faba*) and sesame seed flour [24, 28, 34].
In the case of oxalates, the recorded content was 73.33 ± 0.03 mg per 100 g of dry matter. This is slightly higher than maximum daily intake of 40 to 50 mg per day of oxalate from food recommended by [35]. This proportion is also higher than that of elephant grass (*Eleusine coracana* L.), but remains lower than that of oat bran, wheat bran and red beans [34, 36].

Heavy metal content in dry *Senna occidentalis* dry seeds

Naturally present in environment, heavy metals are essential in trace amounts for many cellular processes within biological tissues, as higher concentrations would make them toxic to body [37]. Results of quantitative analyses of various heavy metals detected in powder of *Senna occidentalis* dry seeds are shown in Table 1 below.

Table 1: Heavy metal content in *Senna occidentalis* dry seeds

Minerals	Contents (mg. kg^{-1})
Lead	0.026
Mercury	0.009
Cadmium	0.010
Arsenic	0.007
Chrome	0.025
Nickel	0.003
Silver	0.001
Cobalt	- 0.043

Note: Negative values indicate concentrations below detection limit of the device (0.0001 ppm).

Looking at this table, we can see that heavy metal content of sample ranged from - 0.043 to 0.003 mg. kg^{-1}. Only lead and chromium showed a somewhat high accumulation capacity. Elements such as mercury, arsenic, nickel and silver were detected at trace levels. Lead was the most abundant element, while cobalt was found in almost negligible quantities. Lead concentration in sample studied here is lower than that found in tomatoes (*Solanum lycopersicon*) and lettuce (*Lactuca sativa*) [38]. Cadmium content is also very low compared to that of cassava (*Manihot esculenta*) [39]. However, these values are all below the tolerated levels for plants in human diet [38].
It is thought that presence and concentrations of heavy metals observed in these dry *Senna occidentalis* seeds are linked to physical and chemical characteristics of soil, as noted by [39] in their work on correlation between metal content in crop soils and metal content in vegetables grown on those soils.

Conclusion

This study highlighted nutritional value of *Senna occidentalis* dry seeds. Their phytochemical and antinutritional factor content and antioxidant activity were determined. They are characterised by a high antioxidant content with high concentrations of phenolic compounds, namely flavonoids and tannins. Their concentration of anti-nutritional factors is low and heavy metals detected are mainly in trace amounts. This potential makes these seeds an interesting resource that can be exploited to achieve an appropriate diet.

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